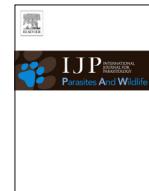




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Toxoplasma gondii exposure in arctic-nesting geese: A multi-state occupancy framework and comparison of serological assays



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ABSTRACT

The zoonotic parasite, *Toxoplasma gondii*, has a worldwide distribution and a cosmopolitan suite of hosts. In arctic tundra regions, the definitive felid hosts are rare to absent and, while the complete transmission routes in such regions have yet to be fully elucidated, trophic and vertical routes are likely to be important. Wild birds are common intermediate hosts of *T. gondii*, and in the central Canadian arctic, geese are probable vectors of the parasite from temperate latitudes to the arctic regions. Our objective was to estimate seroprevalence of *T. gondii* in Ross's and Lesser Snow Geese from the Karrak Lake ecosystem in Nunavut, Canada. After harvesting geese by shotgun, we collected blood on filter paper strips and tested the eluate for *T. gondii* antibodies by indirect fluorescent antibody test (IFAT) and direct agglutination test (DAT). We estimated seroprevalence using a multi-state occupancy model, which reduced bias by accounting for imperfect detection, and compared these estimates to a naïve estimator. Ross's Geese had a 0.39 probability of seropositivity, while for Lesser Snow Geese the probability of positive for *T. gondii* antibodies was 0.36. IFAT had a higher antibody detection probability than DAT, but IFAT also had a higher probability of yielding ambiguous or unclassifiable results. The results of this study indicate that Ross's Geese and Lesser Snow Geese migrating to the Karrak Lake region of Nunavut are routinely exposed to *T. gondii* at some point in their lives and that they are likely intermediate hosts of the parasite. Also, we were able to enhance our estimation of *T. gondii* seroprevalence by using an occupancy approach that accounted for both false-negative and false-positive detections and by using multiple diagnostic tests in the absence of a gold standard serological assay for wild geese.

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1. Introduction

The zoonotic parasite, *Toxoplasma gondii* has a worldwide distribution and a cosmopolitan suite of hosts; evidence of exposure was even recently detected in pinnipeds from Antarctica (Jensen et al., 2012). Oocyst-derived infections are the result of environmental contamination by felids, the definitive hosts of *T. gondii* (Dubey et al., 1970). In arctic tundra regions, felids are rare to absent and, while the complete transmission routes in such regions have

yet to be fully elucidated, trophic routes and transmission from mother to offspring (vertical transmission) are likely to be important (McDonald et al., 1990; Messier et al., 2009).

Wild birds are common intermediate hosts of *T. gondii* (Dubey, 2002). The most common infective forms of *T. gondii* for herbivorous birds, such as geese, are sporulated oocysts, which can be found in contaminated water bodies or soil (Dubey, 2009) to which these birds may be exposed. When high densities of waterfowl congregate in a contaminated environment, oral transmission is likely to occur. If the birds become intermediate hosts of the parasite, they will eventually develop cysts in their organs and musculature. The population-level significance of infection in wild birds is unclear, but avian mortality has been reported in heavily infected birds (Dubey, 2001; Work et al., 2002). Arctic-nesting geese are probable vectors of the parasite from temperate latitudes

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to the arctic region of Svalbard (Prestrud et al., 2007) and likely along other migratory routes as well.

In North America, Ross's Geese (*Chen rossii*) and Lesser Snow Geese (*Chen caerulescens*) are two common arctic-nesting geese that overwinter in the southern United States and migrate through the midcontinent of North America to breeding areas in arctic Canada and Alaska (Alisauskas et al., 2011). Thus, these two populations of arctic-nesting geese are sympatric with both domestic and wild felids for at least 8 months of the year (September to April), but are thought to be largely allopatric to felids for about 4 months (May to August), when in the Arctic. Felid and goose ranges may overlap in the southern portions of the breeding range where about 10% of subarctic geese nest at known colonies near Hudson or James Bay, but 90% nest well above treeline, such as near Queen Maud Gulf, Southampton Island and Baffin Island (Alisauskas et al., 2011); these regions are thought to be largely or completely absent of felid populations. These geese are considered overabundant (Leafloor et al., 2012) because of demonstrated impacts on arctic vegetation (Abraham et al., 2012) from overgrazing (Alisauskas et al., 2012). Such high goose densities across an expanding range represent an increasing potential for seasonal *T. gondii* introduction to wildlife predators in ecosystems of both arctic and temperate latitudes. However, no estimates exist for the seroprevalence of *T. gondii* in these goose populations. Potential predators of geese in the Karrak Lake ecosystem include arctic foxes (*Alopex lagopus*), wolverines (*Gulo gulo*), wolves (*Canis lupus*), and barren-ground grizzly bears (*Ursus arctos horribilis*), and it is possible that infected geese could transmit *T. gondii* to these animals (Bantle and Alisauskas, 1998; Wiebe et al., 2009).

Most evidence for the occurrence of *T. gondii* in wildlife is obtained through serological tests, which, while providing limited information on current infection status, can be useful tools in determining exposure within a population. Filter paper blood collection is a technique that is increasingly used for post-mortem antibody detection in wildlife (Jakubek et al., 2012; Aston et al., 2014). The technique is especially useful in remote areas where sera cannot be refrigerated or frozen, and is commonly used in wild waterfowl (Maksimov et al., 2011). The direct agglutination test (DAT; equivalent to modified agglutination test (MAT)), is a widely used serological test for wildlife exposure to *T. gondii* because it is flexible for use in multiple species and can also be used with eluate from blood stored on filter paper (Jakubek et al., 2012). Although often described as sensitive and specific in wildlife serological applications (Hollings et al., 2013), the DAT has not been formally validated for wildlife and performance can vary among different species (Macrí et al., 2009). Indirect fluorescent antibody tests (IFATs) are also used with wildlife sera (Miller et al., 2002; Dabritz et al., 2008), but their use has been limited to animals for which a taxon-specific secondary antibody has been produced. The use of IFAT with eluate from blood-soaked filter paper is not often reported in *T. gondii* diagnostics, but is commonly used for other types of antibody detection in waterfowl (Maksimov et al., 2011). Both assays have subjective cut-off values based on visual inspection, which suggests the potential exists for misclassification and biased reporting of seroprevalence. In a comparison between IFAT and MAT, Macrí et al. (2009) reported 97.8% sensitivity in cat serum by MAT (with IFAT as the comparative test), but only 73.4% sensitivity in dog serum by MAT. In this case, the IFAT was considered the "gold standard" for comparison by the MAT.

The risk of *T. gondii* transmission from geese to other wildlife populations and people emphasizes the need for reliable parameter estimates from serosurvey data (McClintock et al., 2010). Observation error due to non-detection is not commonly accounted for in prevalence estimates from wildlife disease

studies, although the increasing use of occupancy modeling approaches shows more attention to this concern (e.g., Gómez-Díaz et al., 2010; McClintock et al., 2010, Lachish et al., 2011, Eads et al., 2013). Occupancy approaches are analogous to mark-recapture analyses from wildlife biology and were originally used to estimate the probability of occurrence of cryptic or rare species within habitats where they may be detected imperfectly (MacKenzie et al., 2006). These approaches are useful in wildlife disease ecology because they acknowledge that detection is imperfect and account for this uncertainty in parameter estimates of disease prevalence (McClintock et al., 2010, Lachish et al., 2011). Because most wildlife serological assays are not formally validated and thus have no information on test sensitivity and specificity, occupancy approaches provide a method for quantifying some of the uncertainty in the diagnostic system.

Under a typical occupancy framework, multiple randomly selected 'sites' are repeatedly surveyed within a time frame where the occupancy state (species present or species absent) does not change. Surveys, or replicates, can be conducted at multiple times or at the same time by multiple independent observers or different detection methods. These replicates at each site enable estimation of two parameters: occupancy, defined as the probability that a site is occupied by the species of interest, and detection probability, the probability that the species is detected during a given survey (replicate), given the site is occupied (MacKenzie et al., 2006). In our application, diagnostic samples are 'sites' (i.e., an eluate produced from blood-soaked filter paper taken from each goose) and the species of interest are antibodies against *T. gondii*, and the replicates are repeated assays (DAT or IFAT) performed on each sample.

In more complex occupancy models, such as those handling multiple occupied states or multiple scales, additional parameters can be estimated. Traditional static occupancy models, in a diagnostic context, insulate prevalence estimates against false-negatives but these models assume that false-positive results do not occur. Yet, in all serological assays, and especially with samples from wildlife species, there is a risk of cross-reactivity with unknown non-target antibodies, which could lead to ambiguous test results. Results from the IFAT and DAT are subject to observer experience and opinion, which might cause ambiguous test results to be misclassified, leading to false-positive results. In this paper we utilized a generic multi-state occupancy approach (Nichols et al., 2007) and interpret model parameters in a disease ecology context. A similar approach was also proposed by Miller et al. (2011) and both approaches account for both false-positive and false-negative observational errors.

We hypothesized that Ross's and Lesser Snow Geese are routinely exposed to *T. gondii* because they overwinter in and migrate to areas where *T. gondii* oocysts are likely to be present in the environment. Our main objectives in this study were to: (1) estimate seroprevalence in hunted Ross's and Lesser Snow Geese using a general static multi-state occupancy approach to account for both false-positive and false-negative observational errors, and (2) compare seroprevalence, estimated with occupancy models, to naïve estimates of seroprevalence that assume detection probability is complete and diagnosis is error-free. An additional objective was to evaluate whether species and/or sex had an effect on the probability of seropositivity in a given individual, suggesting apparent differences exist between the species in foraging behavior (Jonsson et al., 2013) or that androgens might suppress immune function, thus leading to increased parasite susceptibility in males (Owen-Ashley et al., 2004). We propose that using different serological assays with multiple replicates and modeling techniques that account for imperfect detection in wildlife samples will reduce bias in estimates of *T. gondii* seroprevalence.

2. Materials and methods

2.1. Field sample collection

Ross's Geese and Lesser Snow Geese were sampled from respective populations by shooting from late May to early June each year, 2011–2013, as part of ongoing studies at Karrak Lake on these species. In both years, geese were collected over a period of 2 weeks and it is unlikely that collected individuals were related. During field necropsies, we collected serosanguineous fluid from the thoracic cavity of each goose on Nobuto filter paper strips (Advantec MFS, Inc, Dublin, CA, USA). The strips were dried at ambient temperature overnight, placed into individual envelopes, and frozen at -20°C until further analysis at the University of Saskatchewan. All field activities were conducted in accordance with The University of Saskatchewan Animal Care and Use Committee (Protocol 20100159), the Canadian Wildlife Service (Permits NUN-SCI-11-02; NUN-MBS-11-03), and the Government of Nunavut (Permits 2011-019, 2012-021, 2013-017).

2.2. Filter paper elution

To facilitate antibody elution from the filter paper strips, we followed the method used by Curry et al. (2011). Briefly, two filter paper strips from each goose were cut into pieces and placed in a microcentrifuge tube. Then, 800 μL Dulbecco's Phosphate Buffered Saline with antibiotic was added to each tube and left to elute in a refrigerator overnight (approximately 16 h). To eliminate debris from dried blood clots on the filter paper, we filtered all eluate samples through a 19-gauge filter needle (Becton Dickenson Canada, Inc., Mississauga, ON) and placed into a new microcentrifuge tube. Eluate samples were stored at -20°C until further analysis.

2.3. Serological analysis

Following a static occupancy-modeling framework, we tested each eluate sample from each goose using three separate replicates of the direct agglutination test (DAT) and two additional replicates using IFAT, resulting in a detection/non-detection sequence for each eluate sample consisting of 5 replicates for which observed antibody states were recorded.

Direct agglutination tests were performed on filter paper eluate using commercially available kits (ToxoScreen-DA, Biomerieux, Marcy l'Etoile, France) according to manufacturer instructions. Each eluate sample was tested in duplicate wells at 1:40 dilution on three discrete occasions. Test wells indicating agglutination covering 50–100% of the well were recorded as "*T. gondii* antibodies clearly detected". Following manufacturer's instructions, test wells with a solid dot or small ring in the center were recorded as "no antibodies detected", and wells with mild agglutination covering less than half of the test well were recorded as "ambiguous or unclassifiable".

Indirect fluorescent antibody tests (IFATs) were performed using anti-duck fluorescein isothiocyanate (FITC; rabbit origin) conjugate from Nordic Laboratories (Copenhagen, Denmark) and antigen-coated slides from VMRD (Pullman, WA, USA). The IFAT was optimized using *T. gondii*-positive and negative control filter paper samples from experimentally infected and unexposed control Pekin ducks (S.A. Elmore, unpublished data), concluding that the assay best performed at both a sample dilution and conjugate dilution of 1:20. The staining procedure followed manufacturer's (VMRD) instructions. We scanned the slides by fluorescent microscopy at $40\times$ magnification. Sample wells with unbroken staining surrounding the entire tachyzoite were recorded as "*T. gondii* antibodies clearly detected". Sample wells with little or no staining, or

where tachyzoites in the well demonstrated discontinuous staining were recorded as "no antibodies detected"; sample wells with dim fluorescence and both intact and discontinuous staining were recorded as "ambiguous or unclassifiable".

2.4. Data analysis

We calculated naïve seroprevalence by dividing the number of geese that were clearly *T. gondii* seropositive on at least one replicate, of either assay, by the total number of samples tested.

To utilize the general multi-state occupancy model (Nichols et al., 2007) we first describe three true states; each eluate sample must be in one of these mutually exclusive states: (i) Eluate sample contains no antibodies that may lead to non-negative assay results (True state = 0), (ii) Eluate sample contains non-target antibodies or other material that may lead to an ambiguous or unclassifiable assay result (True state = 1), and (iii) Eluate sample contains targeted *T. gondii* antibodies (True state = 2). The multi-state occupancy model contains two occupancy parameters, ψ^1 and ψ^2 , that are used to estimate the probability that a given eluate sample is in each of the three true states (see Table 1). We utilized our observed detection histories for antibodies results for each replicate assay with the static multiple-state occupancy model implemented in Program MARK (White and Burnham, 1999) to estimate the two occupancy parameters and derive seroprevalence for our sampled goose populations ($\psi^1 \times \psi^2$; Table 1). In addition, the multi-state occupancy model contains two detection probability parameters (p^1 and p^2 ; see Table 1), and a correct classification parameter (δ ; see Table 1). Here, p^1 represents the probability of an ambiguous or unclassifiable assay result even though the eluate does not contain *T. gondii* antibodies. We assume that an eluate without *T. gondii* antibodies cannot produce an unambiguous positive result (i.e., the "*T. gondii* antibodies clearly detected" result as defined above). Notice that an eluate that contains *T. gondii* antibodies can produce an assay with any of the three possible observations: *T. gondii* antibodies are clearly detected (probability = $p^2 \times \delta$), ambiguous or unclassifiable results (probability = $p^2 \times (1-\delta)$), or non-detection results (probability = $(1 - p^2)$).

Using our multi-state occupancy model, we developed an *a priori* candidate model set (Table 2) to examine the effects of sex and species on the probability of *T. gondii* seropositivity, and the effect of serological method (DAT vs IFAT) on both detection probabilities (p^1 and p^2) and the correct classification probability (δ). We performed model selection using small sample bias-corrected Akaike Information Criterion values (AICc; Burnham and Anderson, 2002). We ranked candidate models by calculating the differences in AICc (ΔAICc) between the highest-ranked model and the other models, and then calculated model weights (w_i) for each model; higher-ranked models carry the most weight and thus best explain the data (Anderson, 2008). Because most of the weight was distributed between the two highest-ranked models, we addressed model selection uncertainty by performing model averaging to obtain final parameter estimates (Anderson, 2008).

3. Results

We analyzed blood filter paper eluent by DAT and IFAT for *T. gondii* antibodies in 121 Lesser Snow Geese and 123 Ross's Geese from Karrak Lake Nunavut. We detected *T. gondii* antibodies in samples from both species (Fig. 1). Our occupancy-based model-averaged estimates of seroprevalence in geese were higher than those calculated using a naïve approach (Fig. 1). The occupancy seroprevalence estimate for Ross's Geese was 0.39 (95% CI = 0.27, 0.51) while the naïve estimate was 0.26. In Lesser Snow Geese, results were similar, with an occupancy estimate of 0.36 (95%

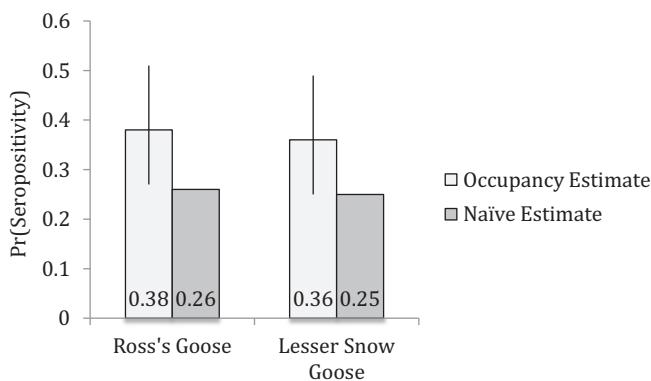
Table 1

Definition of three mutually exclusive true states and associated multi-state occupancy model parameters.

True state	Definition	Probability
0	Eluate contains no antibodies that may lead to a non-negative assay result	$(1 - \psi^1)$
1	Eluate contains non-target antibodies or other materials that may lead to a non-negative assay result	$\psi^1 \times (1 - \psi^2)$
2	Eluate contains target <i>T. gondii</i> antibodies	$\psi^1 \times \psi^2$
Parameter	Definition	
ψ^1_i	Probability that an eluate sample i contains antibodies (non-target or <i>T. gondii</i>) or other materials that may yield a non-negative assay result.	
	Probability (true state = 1 or 2)	
ψ^2_i	Probability that an eluate sample i is occupied by <i>T. gondii</i> antibodies, given a non-negative result is possible. Probability (true state = 2 true state = 1 or 2)	
$\psi^1_i \times \psi^2_i$	Unconditional probability that sample i contains <i>T. gondii</i> antibodies. Probability (true state = 2)	
p_{it}^1	Probability of an ambiguous or unclassifiable result from assay t , given the eluate sample i contains only non-targeted antibodies (i.e., the sample is in true state 1)	
p_{it}^2	Probability of an ambiguous or clear positive result from assay t , given <i>T. gondii</i> antibodies are in eluate sample i (i.e., the sample is in true state 2)	
δ_{it}	Probability that a non-negative result from assay t is correctly classified as <i>T. gondii</i> seropositive, given <i>T. gondii</i> antibodies are in eluate sample i (i.e., the sample is in true state 2)	

Table 2Candidate model set and model selection results of multi-state occupancy analysis to determine seroprevalence, detection probability and classification probability of *T. gondii* antibodies in wild geese from Karrak Lake, Nunavut, Canada.

Model	No. parameters	-2 Log likelihood	AICc	Δ AICc	AICc weight
$\psi^1(\text{species}) \psi^2(\text{species}) p^1(\text{test}) p^2(\text{test}) \delta(\cdot)$	9	1180.73	1199.50	0.00	0.62
$\psi^1(\text{species}) \psi^2(\text{species}) p^1(\text{test}) p^2(\text{test}) \delta(\text{test})$	10	1179.77	1200.71	1.21	0.34
$\psi^1(\cdot) \psi^2(\cdot) p^1(\text{test}) p^2(\text{test}) \delta(\cdot)$	7	1191.83	1206.30	6.81	0.02
$\psi^1(\cdot) \psi^2(\cdot) p^1(\text{test}) p^2(\text{test}) \delta(\text{test})$	8	1190.72	1207.33	7.83	0.01
$\psi^1(\text{sex}) \psi^2(\cdot) p^1(\text{test}) p^2(\text{test}) \delta(\cdot)$	9	1189.85	1208.61	9.11	0.01
$\psi^1(\text{sex}) \psi^2(\cdot) p^1(\text{test}) p^2(\text{test}) \delta(\text{test})$	10	1188.46	1209.40	9.90	0
$\psi^1(\text{species}) \psi^2(\text{species}) p^1(\cdot) p^2(\cdot) \delta(\cdot)$	7	1275.35	1289.82	90.32	0
$\psi^1(\text{species}) \psi^2(\text{species}) p^1(\cdot) p^2(\cdot) \delta(\text{test})$	8	1275.18	1291.79	92.29	0
$\psi^1(\cdot) \psi^2(\cdot) p^1(\cdot) p^2(\cdot) \delta(\cdot)$	5	1285.49	1295.74	96.24	0
$\psi^1(\cdot) \psi^2(\cdot) p^1(\cdot) p^2(\cdot) \delta(\text{test})$	6	1285.35	1297.70	98.20	0
$\psi^1(\text{sex}) \psi^2(\text{sex}) p^1(\cdot) p^2(\cdot) \delta(\cdot)$	7	1284.50	1298.98	99.48	0

**Fig. 1.** Comparison of seroprevalence estimates for Ross's Geese and Lesser Snow Geese generated by naïve and multi-state occupancy estimators (seroprevalence = $\psi^1 \times \psi^2$).

$CI = 0.25\text{--}0.49$) and a naïve estimate of 0.25. Naïve seroprevalence estimates were either below or equivalent to the lower bound of the model-based confidence intervals. Our results indicate that naïve values using only a single assay would have been well below estimated seroprevalence estimates for both species.

Host species and assay type were found to be associated with the probability of seropositivity and detection, respectively (Tables 2 and 3). Model-averaged estimates suggested that Ross's geese were slightly more likely to be seropositive than Lesser Snow Geese, but 95% confidence intervals showed considerable overlap (Table 3) with little difference in effect size. IFAT had a higher

Table 3

Model-averaged parameter estimates of seroprevalence, detection probability, and classification.

Parameter	Estimate	SE	95% LCI	95% UCI
$\psi^1_{\text{Female SNGO}} \times \psi^2_{\text{Female SNGO}}$	0.36	0.06	0.25	0.49
$\psi^1_{\text{Male SNGO}} \times \psi^2_{\text{Male SNGO}}$	0.36	0.06	0.25	0.49
$\psi^1_{\text{Female ROGO}} \times \psi^2_{\text{Female ROGO}}$	0.39	0.06	0.27	0.51
$\psi^1_{\text{Male ROGO}} \times \psi^2_{\text{Male ROGO}}$	0.39	0.06	0.27	0.51
$\psi^1_{\text{Female SNGO}}$	0.99	*	*	*
$\psi^1_{\text{Male SNGO}}$	0.99	*	*	*
$\psi^1_{\text{Female ROGO}}$	0.55	0.15	0.28	0.80
$\psi^1_{\text{Male ROGO}}$	0.55	0.15	0.28	0.80
$\psi^2_{\text{Female SNGO}}$	0.36	0.08	0.23	0.53
$\psi^2_{\text{Male SNGO}}$	0.36	0.08	0.23	0.52
$\psi^2_{\text{Female ROGO}}$	0.71	0.17	0.32	0.92
$\psi^2_{\text{Male ROGO}}$	0.71	0.17	0.32	0.93
(p_{DAT}^1)	0.01	*	*	*
(p_{IFAT}^1)	0.17	0.04	0.10	0.27
(p_{DAT}^2)	0.21	0.03	0.16	0.28
(p_{IFAT}^2)	0.54	0.05	0.44	0.64
(δ_{DAT})	0.55	0.06	0.43	0.67
(δ_{IFAT})	0.58	0.06	0.47	0.68

* Parameter estimated at boundary of parameter space. Standard error cannot be estimated.

probability of detecting antibodies than DAT, either in the presence of *T. gondii* antibodies (p^2) or when only non-target antibodies or other material was present (p^1). The DAT test rarely resulted in an ambiguous detection when *T. gondii* antibodies were absent in the eluate sample ($\hat{p}_{\text{DAT}}^1 = 0.01$), but given that *T. gondii* antibodies were present, the probability that this test resulted in a clear positive detection was only 0.12 ($\hat{p}_{\text{DAT}}^2 \times \delta_{\text{DAT}}$). Conversely, while IFAT was more likely to produce ambiguous or unclassifiable results

(Table 3: $\hat{p}_{IFAT}^1 = 0.17$ and $\hat{p}_{IFAT}^2 \times (1 - \hat{\delta}_{IFAT}) = 0.23$), the probability that the IFAT test produced a clear positive detection, given that the goose sample was positive for *T. gondii* antibodies, was almost 3 times higher than for the DAT assay ($\hat{p}_{IFAT}^2 \times \hat{\delta}_{IFAT} = 0.31$).

4. Discussion

Our study suggests that both populations of Ross's and Lesser Snow Geese sampled from the central Canadian Arctic were exposed to *T. gondii* at some point in their lives, supporting the hypothesis that waterfowl can be a source of *T. gondii* introduction in the terrestrial Canadian Arctic. Although *T. gondii* antibodies have been detected in other species of wild goose (Prestrud et al., 2007; Murao et al., 2008; Sandström et al., 2013), to our knowledge this study is the first to document seropositive Ross's and Lesser Snow Geese. *T. gondii* exposure in Ross's and Lesser Snow Geese probably occurs on wintering grounds in the southern United States and along migratory flyways, where they feed in agricultural fields at numerous stopover points in North America (Alisauskas et al., 1988; Alisauskas and Ankney, 1992). We did not test blood filter paper samples from juvenile geese that had not yet migrated south, thus we cannot rule out possible exposure to *T. gondii* on the nesting grounds at Karrak Lake, Nunavut. However, Sandström et al. (2013) did not detect any antibodies in juvenile geese from Arctic brood-rearing locations on Svalbard, suggesting that these animals were exposed solely at temperate latitudes. *T. gondii* antibodies were detected in adult geese, however, indicating that geese are exposed after migrating from the breeding ground. A survey of hatch-year geese on the brooding grounds in the Queen Maud Gulf Bird Sanctuary would help determine whether geese are exposed to *T. gondii* while in the Canadian central arctic.

Few studies report the seroprevalence of *T. gondii* in wild geese from North America. Dubey et al. (2014) reported the detection of *T. gondii* antibodies in 2 of 2 Canada Geese (*Branta canadensis*) from Pennsylvania, USA. The occurrence of *T. gondii* in other species sympatric with Ross's and Lesser Snow Goose wintering grounds is unknown. In Europe, Prestrud et al. (2007) reported a 7% sample seroprevalence in Barnacle Geese (*Branta leucopsis*) from Svalbard. Sandström et al. (2013) reported seroprevalence rates of 6.5% in Pink-Footed Geese (*Anser brachyrhynchus*) from Svalbard, and 25% in migratory Barnacle Geese on wintering grounds in the Netherlands. Neither of these studies accounted for potential false-positive or false-negative errors.

Sex of goose hosts did not influence seroprevalence, but we found support for species-specific differences in model parameters (Table 2). Our estimates suggest than nearly all samples collected from Snow Geese contain antibodies or other material that may lead to non-negative assay results ($\hat{\psi}_{SNGO}^1 = 0.99$), while only 55% of samples from Ross's Geese had these antibodies. Given that antibodies or similar material existed in a sample, Ross's Geese were much more likely to contain *T. gondii* antibodies ($\hat{\psi}_{ROGO}^2 = 0.71$) than Snow Geese ($\hat{\psi}_{SNGO}^2 = 0.36$).

Ross's Geese might be more likely to be exposed to *T. gondii* oocysts than Lesser Snow Geese due to differences in feeding ecology. Ross's Geese have a smaller bill that is better suited for grazing in pastures and short tundra grasses, whereas the larger Snow Goose is known to grub in the soil for roots and tubers (Alisauskas et al., 1988; Jonsson et al., 2013). Presence of *T. gondii* oocysts might be more likely on shoots of vegetation than on below-ground portions of plants or in the soil, suggesting that differences in feeding mechanism could explain the differences in parameter and seroprevalence estimates we observed.

Lesser Snow Geese and Ross's Geese are commonly hunted waterfowl species throughout central North America, along the Pacific Flyway, and in their arctic summer habitat. The

seroprevalences (36% and 39%, respectively) of *T. gondii* in these species demonstrate the potential for geese to transmit infection to predator animals, hunters and people who process the carcasses of hunted geese. However, current food preparation practices in the central Canadian Arctic might already be protective; goose meat is commonly boiled, following a specific carcass-handling procedure (D. Stern, personal communication). In other areas of the Canadian North, goose meat is smoked and barbecued (Ohmagari and Berkes, 1997). Thoroughly boiling and otherwise cooking *T. gondii*-infected meat at 60 °C or higher will kill tissue cysts (Dubey, 2009). The viability of cysts after drying is unclear and probably variable; however Lundén and Uggla (1992) did not recover infective *T. gondii* from mutton that was cured, smoked, or frozen. Also, cats fed sausage, igunaq (fermented muscle), and nikku (dried muscle) from experimentally infected seals (*Halichoerus grypus*) did not shed oocysts after exposure (Forbes et al., 2009). Sanitary measures during processing and preparing meat, and thorough cooking of meat (if culturally acceptable) before consumption would help reduce transmission of *T. gondii* to people (Kapperud et al., 1996).

We compared seroprevalence estimates using occupancy-based and naïve estimation methods. The occupancy approach demonstrated a higher estimated seroprevalence in both goose species than the naïve estimators. From the difference between types of estimates, it is clear that failure to account for detection probability results in an underestimate of seroprevalence, and thus could result in an underestimation of infection status in wild geese.

When comparing the two assays used in this study, the IFAT test seemed to outperform the DAT when used on wild goose filter paper eluate samples, because it showed both a higher probability of *T. gondii* antibody detection and a higher probability of correct classification of serology conditional on the antibody being present. The higher detection probability of IFAT reflects more consistency in the test across the multiple replicates than in the DAT. However, IFAT also resulted in a higher probability of unclassifiable or ambiguous serological results when only not-target antibodies are present (True state = 1), which could lead to bias in serological studies if false-positive errors are not considered in the analysis. The high occurrence of unclassifiable or ambiguous results might reflect inaccurate optimization of the IFAT assay, in which case more replicates of known positive and negative animals might improve assay validity. Filter paper eluent is variable in nature and differences will exist in sample handling between filter paper from experimentally infected versus free-ranging animals. These factors, plus the differences between laboratory samples and field-collected samples might have contributed to uncertainty in the IFAT results. The low false-positive (p^1) and true-positive (p^2) detection probabilities estimated with the DAT indicates that a simple occupancy-modeling framework (MacKenzie et al., 2006) is ideal for this assay, as repeated sampling should provide a more precise estimate of true detections or non-detections. Study designs that rely only on one DAT repetition are more likely to underestimate the seroprevalence, and thus lead to biased inference about occurrence of *T. gondii* infection within the study population.

Both DAT and IFAT performed similarly in correctly classifying positive results, which indicates that if *T. gondii* antibodies exist and are detected, the result is categorized correctly 55–58% of the time. Given the different types of uncertainty introduced by each test, we recommend parallel testing with both DAT and IFAT assays to improve the sensitivity of seroprevalence estimates in waterfowl. Although DAT demonstrated a much lower antibody detection probability than IFAT, it also had a much lower probability for unclassifiable results, suggesting that the DAT results provide less ambiguous estimates of *T. gondii* seroprevalence, provided that one accounts for non-detection (false negative

errors). However, the tradeoff is that, because DAT appears to detect antibodies in fewer seropositive samples, these estimates are likely to be biased if DAT is used alone without accounting for non-detection. Moreover, lower detection probability associated with any method would reduce the precision of the estimate of seroprevalence, the parameter of main interest.

Both assays used in this study demonstrated strengths and weaknesses in detection ability. The IFAT and DAT diagnostic tests used in this study have positive/negative cut-off characteristics that are inherently subjective, thereby increasing the risk of violating traditional occupancy modeling assumptions of no observational error. With a multi-state occupancy approach, we were able to estimate the seroprevalence of *T. gondii* in wild geese while accounting for both false positive and false negative results. Also, we were able to gather information about the uncertainty of both assays through the use of equivocal test results, which might ordinarily be discarded during a traditional serological analysis.

Sample collection in remote and arctic wildlife systems is both logistically and physically challenging. Reducing bias in seroprevalence estimates through thoughtful study design and rigorous data analysis, such as with repeated tests and an occupancy analysis approach, can increase the quality of serosurveys to match the effort required. Wildlife researchers might reduce uncertainty in future serosurveys if repeat testing is performed, if the data analysis accounts for imperfect detection, and if multiple assays are used. Multiple analytical models can be used to accommodate this type of data to provide unbiased estimates of seroprevalence (Nichols et al., 2007; Miller et al., 2011) and are readily available in various, free programs (e.g., programs MARK and Presence).

The potential for *T. gondii* to impact wildlife health and reproduction and the risk of transmission from geese to people and predators such as arctic canids and subarctic felids emphasizes the need for robust estimates of parasite prevalence. Because serosurveys only indicate exposure and not true infection, future work could focus on testing tissues of arctic-nesting geese to determine in which organ the parasite can be most easily detected and molecular characterization of arctic isolates of *T. gondii*. Such research will provide information about how the parasite is introduced and maintained in terrestrial Arctic ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijppaw.2014.05.005>.

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